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SHORT COMMUNICATION

Analysis of Genetic Diversity in *Alpinia galanga* using ISSR Markers

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Genetic diversity analysis was carried out in *Alpinia galanga* accessions collected from Kerala state of India using Inter Simple Sequence Repeats (ISSR) markers. A total of 60 bands were generated from 18 accessions of *A. galanga* using 11 ISSR primers. Of these 24 (40%) were polymorphic and remaining 36 (60%) were monomorphic. Total Nei's genetic diversity among the three populations was estimated as h= 0.1735 $\pm$ 0.013 and I= 0.2470 $\pm$ 0.078. Nei's estimator of population substructure (G_{ST}) indicated a fairly low level of population differentiation (G_{ST} = 0.2139). Based on this study we propose a conservation strategy to this species.

Key Words: *Alpinia galanga*, Genetic differentiation, Genetic diversity, Greater galangal, ISSR

Alpinia galanga (Linn.) Willd. (family Zingiberaceae) is an important medicinal-cum-aromatic plant distributed in India, Indo-China, Philippines and Borneo (Sabu, 2006). It is commonly called as Greater galangal or *Valia-aratha*. It is a clonally propagated species and is sparsely represented in western ghats of India (Sabu, 2006). The aromatic rhizome of *A. galanga* is extensively used in traditional medicines like Ayurvedic, Unani, Chinese, and Thai folk systems to cure diseases such as bronchitis, diabetes, heart diseases and kidney disorders. It is also effective to alleviate pain specifically head-ache, chest pain and rheumatic pain. The rhizome extract has the potential to stimulate digestion, purify blood and to improve voice (Sabu, 2006). It has also been listed among the promising cancer preventing dietary supplements (Ohigashi, 2002). Raw rhizome is an essential ingredient in many Indonesian and Malaysian dishes for its ginger-like flavour.

Genetic study of clonal plant species provides reliable information on their population dynamics and detailed demographic data (Kareem *et al*., 2012; Rajasekharan and Kareem, 2010). To the best of our knowledge, genetic characterization of *A. galanga* is insufficient to propose a suitable conservation strategy (Saritnum and Sruamsiri, 2003). The present study focussed on the genetic diversity analysis in *A. galanga* accessions collected across Kerala state of India using Inter Simple Sequence Repeats (ISSR) markers.

ISSR amplifies inter-microsatellite sequences at multiple loci throughout the genome (Rajasekharan and Kareem, 2010). These markers offer a great potential to determine intra- and inter-genomic diversity compared with other arbitrary primers, since these reveal variations within unique regions of the genome at multiple loci simultaneously. ISSRs have become popular and effective markers for measuring genetic diversity in clonal plants as-well-as in medicinal plants (Kareem *et al*., 2011, 2012; Mohan *et al*., 2013; Rajasekharan and Kareem, 2010, 2015).

A survey was conducted to collect *A. galanga* populations in Kerala state of South India. A total of eighteen accessions were collected from different locations along with their geographical coordinates. From the study, three populations were identified *viz*., Trivandrum zone, Cochin zone and Calicut zone. The altitudes of the collection sites were ranged from 17 m to 204 m.

Genomic DNA was extracted from fresh leaves (0.5g) of *A. galanga* using modified CTAB method (Sane *et al*., 2012). ISSR-PCR reaction was performed in a volume of 10 µl containing 50 ng template DNA, 0.5mM dNTPs (Chromous Biotech, Bangalore, India), 0.15U *Taq* DNA

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polymerase (Chromous Biotech), 0.5µM ISSR primers from University of British Columbia (The Michael Smith Laboratories, University of British Columbia, primer set # 9, Vancouver, BC, Canada) and 1X PCR buffer (10mM Tris HCl pH 8.3, 50mM KCl, 3mM $MgCl_2$) (Merck). A total of eleven reproducible primers were selected from 100 ISSR primers for the present study based on the consistent banding pattern (Table 1). The reactions were carried out in a DNA thermocycler (Eppendorf mastercycler gradient, Germany) with an initial denaturation step of 94°C for 4 min, followed by 34 cycles at 94°C for 1 min, 45 sec at the specific annealing temperature of each primer (44 to 56°C) and 72°C for 1 min and a final extension at 72°C for 8 min and a hold temperature of 4°C at the end. After amplification, the reaction products were subjected to electrophoresis in 1.5% agarose gels in 1x TAE buffer stained with 5µg ml^{-1} ethidium bromide and photographed under UV light with the help of a gel documentation system (Syngene). 1Kb molecular ladder was used as marker to know the size of the fragments.

Amplified products, which were reproducible and consistent in performance, were scored for band presence (1) or absence (0) and a binary qualitative data matrix was constructed. Percentage of polymorphic bands (PPB) was calculated by dividing the number of polymorphic bands by the total number of bands surveyed. The binary matrix was used to determine the genetic diversity, genetic differentiation and gene flow using the software PopGene version 1.31 (Yeh *et al.*, 1999). Genetic diversity within and among populations were measured by PPB, effective number of alleles (ne), observed number of alleles (na), Nei's (1973) gene diversity (*h*) and Shannon's information index (*I*). At the species wide level, total genetic diversity (H_T) and genetic diversity within populations (H_S) were calculated. To estimate the genetic divergence among populations, we also calculated the relative magnitude of genetic differentiation among populations ($G_{ST} = (H_T - H_S)/H_T$). Corresponding estimates of gene flow (*N*m), *i.e.* the average per generation number of migrants exchanged among populations, was estimated using the formula: $Nm = 0.5(1 - G_{ST})/G_{ST}$.

In addition, an analysis of molecular variance (AMOVA) was performed to calculate the partitioning of genetic variance among and within population using GenAlEx ver. 6.41 (Peakall and Smouse, 2006). The permutation number for significance testing was set to 999 for all the analysis. To explore the genetic relationships among all populations, a UPGMA (Unweighted Pair-Group Method using Arithmetic average) dendrogram was constructed based on the matrix of Nei's genetic distance by the program TFPGA, version1.3. Given that the above estimation of allele frequencies from dominant markers requires the assumption of Hardy–Weinberg equilibrium.

Table 1. Details of ISSR primers used for PCR amplification of *A. galanga**

S.No.	Primer No	Primer sequence	Total number of bands obtained	Percentage of Polymorphic bands
1	811	$(GA)_8C$	3	66.6
2	814	$(CT)_8A$	5	60
3	827	$(TC)_8A$	4	50
4	834	$(AG)_8YT$	5	40
5	844	$(CT)_8RC$	3	33.3
6	855	$(AC)_8YT$	5	40
7	857	$(AC)_8YG$	5	40
8	866	$(CTC)_6$	6	33.3
9	873	$(GACA)_4$	3	33.3
10	887	DVD $(TC)_7$	10	30
11	902	CTC $(GT)_8$	5	40
Total / Av.			60	41.66

*D = (A, G, T); R = (A, G); Y = (C, T).

A total of 60 bands were generated from 18 accessions of *A. galanga* by using 11 ISSR primers (Fig. 1). Of these 24 (40%) were polymorphic and remaining 36 (60%) were monomorphic. Number of bands varied between 3-10 and average 5 bands per primer was observed (Table 1). Total Nei's genetic diversity among the three populations was estimated as $h = 0.1735 \pm 0.013$ and $I = 0.2470 \pm 0.078$ (Table 2). South Kerala population exhibited more genetic diversity (PPB= 40%; $h = 0.1699 \pm 0.071$; $I = 0.2437 \pm 0.10$) than the other two populations. Average genetic variation within population was observed as $h = 0.1364 \pm 0.009$. The observed and effective number of alleles across the populations were $na = 1.4 \pm 0.11$ and $ne = 1.32 \pm 0.11$ respectively. The current study reveals a relatively a low level genetic diversity exist within and among populations of *A. galanga*. The low genetic diversity may be due to high rhizome distribution as a clonal propagule.

Across the three populations of *A. galanga* surveyed for ISSR variation, Nei's estimator of population substructure (G_{ST}) indicated a fairly low level of population differentiation ($G_{ST} = 0.2139$) (Table 2). These G_{ST} values translated into correspondingly

Table 2. Analysis of genetic diversity in *A. galanga* population detected by ISSR*

Population	h	I	PPB	G_{ST}	Nm
Trivandrum zone	0.1699 ± 0.071	0.2437 ± 0.10	40%		
Cochin zone	0.0923 ±0.091	0.1328 ± 0.12	21.67%		
Calicut zone	0.1470 ± 0.124	0.2100 ± 0.17	33.33%		
Mean	0.1364 ± 0.009	0.1955	31.66%		
Species level	0.1735 ± 0.013	0.2470 ± 0.078	40%	0.2139	1.8375

**h*, Nei's (1973) diversity index; *I*, Shannon's information index; PPB, percentage of polymorphic loci; G_{ST}, genetic differentiation between populations (Nei's); *N*m, estimated

high levels of gene flow (*Nm*), with 1.8375 migrants exchanged between populations (on average) each generation. The AMOVA also revealed that all of the genetic variation exists within the populations rather than among the populations. The dendrogram obtained using the UPGMA algorithm based on Nei's genetic distance is presented in Fig. 2. The analysis showed that populations of Trivandrum zone and Calicut zone exhibited more closeness whereas those of Cochin zone was little diverged from the two.

The information on genetic diversity and relationship within and among crop species is essential for the efficient utilization of plant genetic resource collections (Rajasekharan and Kareem, 2010). Our study is concerned with the evaluation of the genetic diversity and relationship of *A. galanga*. It could be explained that *A. galanga* has been concurrently moved across vast geographic locations within India; therefore, genetic differentiation of *A. galanga* has been affected. Genetic improvement of *A. galanga* should be based on molecular variation as well as morphological differences. Our investigation of genetic diversity by ISSR markers clearly showed that there is low genetic variation within and among *A. galanga* populations in Kerala. Similar result was observed in some clonally propagated species, such as *Alternanthera philoxeroides* (Wang *et al*., 2005). In general, clonally propagating plant species exhibit low genetic variation within and among population. It is widely accepted that population genetic variation is influenced by factors, such as historical events, breeding system, genetic drift and natural selection. Relatively low genetic diversity in *A. galanga* may be related to its introduction or cultivation history. It is thought that, from the present study, the source material for the Kerala population might have come from single propagule or from closely related propagules. *A. galanga* usually propagates through its rhizome which makes genetically uniform population.

The population genetic structure of a species is affected by a number of evolutionary factors including mating system, gene flow, extent of population diversity as well as natural selection (Hamrick and Godt, 1990). The clonal propagation theoretically has similar effects for population genetic structure as strict selfing (inbreeding). Inbreeding mode usually reduces gene exchange (gene flow) both between different individuals

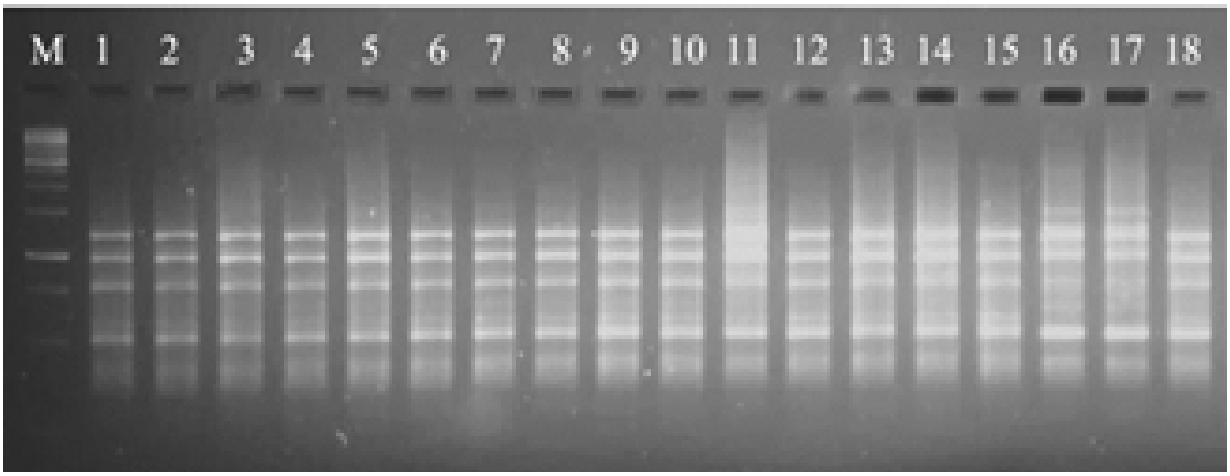

Fig 1. ISSR profile of 18 accessions of *A. galanga* Lane M- DNA marker, Lanes 1 to 18 ISSR profiles of individual accessions of *A. galanga*

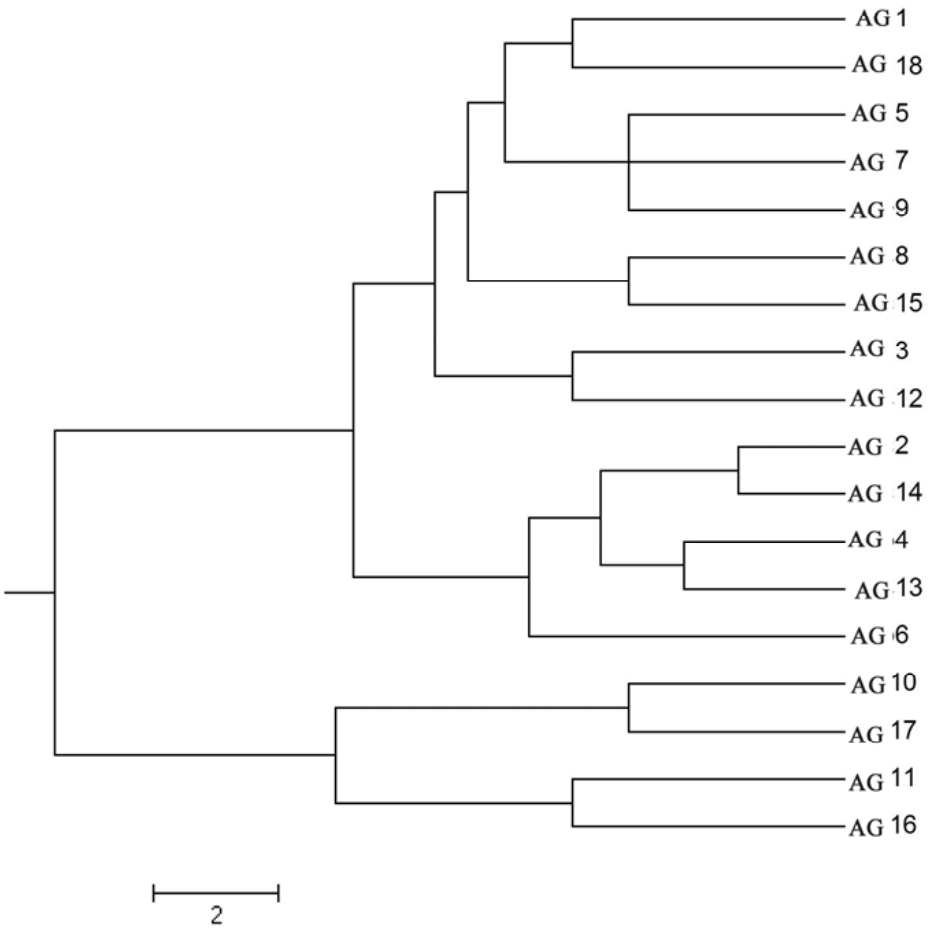

Fig. 2. UPGMA dendrogram showing genetic relationship between 18 accessions of *A. golanga* based on 60 ISSR markers

and populations, leading to significant differentiation between populations. The value of G_{ST} observed in the present study was much lower than the average G_{ST} for selfing species (0.51 (Hamrick and Godt, 1990)). This is thought to be due to the reproductive mode of *A. galanga*.

Could the cultivation practices result in the homogeneity or decrease of genetic diversity after several decades of cultivation? This question becomes more important while wild gene pools of this species decreased rapidly and lack of good cultivars. Clonal reproduction does not involve recombination and, therefore, yields offspring that are genetically identical to each other and to the mother plant. The extent of genetic diversity of *A. galanga* in cultivated populations in present studies was lower as in the case of any clonally propagated plant. Genetic differentiation and gene flow are important index to evaluate the population genetic structure of a species. Gene flow, the movement of gene within and between populations, is negatively correlated with genetic differentiation, but is very important for population transfer and plant evolution. Conservation of wild plants has a major focus on preserving viability of existing populations through *in situ* programme. Since realization of importance of *ex situ* collections for conservation *in situ*, limitations of their utility became evident.

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